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Research Concept: Isoflavones in Soy Infant Formula

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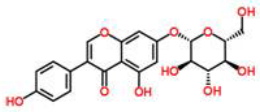
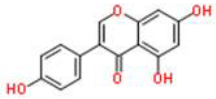
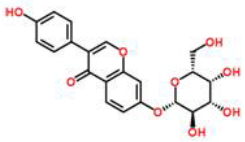
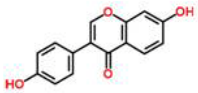
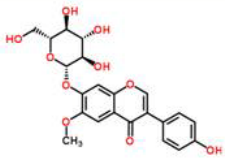
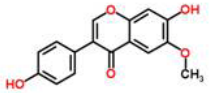
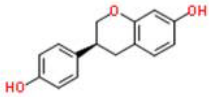
May 10, 2010
NTP Board of Scientific Counselors

Isoflavones in Soy Infant Formula

Sugar-bound isoflavones (not biologically active)		Aglycone isoflavones (biologically active)	
Genistin MW: 423.37 58-66% of isoflavone content		**Genistein** MW: 270.24	
Daidzin MW: 416.37 29-34% of isoflavone content		**Daidzein** MW: 254.24	
Glycitin MW: 446.41 5-8% of isoflavone content		**Glycitein** MW: 284.26	
Relative in vitro estrogenic potency: genistein ≈ equol > daidzein > glycitein		**Equol** MW: 242.27	

Background

- On March 16, 2010, NTP released draft NTP Brief on Soy Infant Formula expressing "minimal concern" for adverse effects on human development
- The draft NTP conclusion of "minimal concern" was based on:
  - Clear evidence for adverse effects of genistein on reproductive development and function in female rats and mice manifested as
    - accelerated puberty (i.e., decreased age at vaginal opening)
    - abnormal estrous cyclicity
    - cellular changes to the female reproductive tract
    - decreased fecundity (i.e., decreased fertility, implants, and litter size)
  - Blood levels in human infants fed soy infant formula can exceed those measured in neonatal or weanling rodents following treatment with genistein at dose levels causing adverse effects in the rodents

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Key Data Gaps in the Animal Literature

Potential Effects of Isoflavones and Non-Isoflavone Components of Soy Infant Formula

- Majority of studies have evaluated the effects of only one isoflavone: genistein
- Other soy isoflavones may influence the action of genistein
- Non-isoflavone components may affect the absorption or biological activity of the isoflavone components
  - Corn syrup, vegetable oils, sugar, vitamins, minerals and other nutrients
  - Contaminants include:
    - Phytates (bind minerals and niacin)
    - Protease inhibitors (have antitrypsin, antichymotrypsin, and antielastin properties)
    - Minerals added to compensate for phytate binding and heated to inactivate protease inhibitors

Too Few Period of Lactation-Only Studies with Soy Infant Formula or Soy Isoflavone Mixtures

- Only 2 studies of soy infant formula considered of "limited" utility
  - Female not assessed in marmoset studies (Sharpe 2002; Tan 2006)
- Many studies of soy diet, soy protein isolate, or mixtures of soy isoflavones included treatment outside the period of lactation
  - Considered of "limited" utility at most by expert panel
- Period of lactation-only studies of soy diet, soy protein isolate, or mixtures of soy isoflavones provided "insufficient" evidence to reach a conclusion in draft NTP Brief on Soy Infant Formula
  - Findings often inconsistent
  - Difficult to reconcile due to variations in experimental design, administered form of soy product, dose levels tested, and treatment protocols

Sharpe (2002) Hum Reprod 17(7): 1692-1703; Tan (2006) Hum Reprod 21(4): 896-904.

Issue of Equol Production in Rodents and its Relevance to Human Infants

- Issue is the contribution of equol to the *in vivo* estrogenic potency of daidzein
  - *In vitro* estrogenic potency of equol is similar to genistein
  - 30-50% of adult humans, and even fewer infants, considered "equol producers"
  - Adult rodents and non-human primates produce equol; no information on rodent pups
- Some suggestion that estrogenicity of equol *in vivo* less than predicted based on *in vitro* studies
  - Attributed to significant conjugation of equol in blood
    - Equol detected in infant and adult rhesus monkeys treated with daidzein but < 0.3% was detected in unconjugated form (Dan Doerge, NCTR/FDA, personal communication)

Specific Aims:

Using period of lactation-only, oral dosing of mouse or rat pups:

1. Identify the developmental profile of daidzein metabolism to equol during development
2. Evaluate how individual soy isoflavones act in combination on an estrogen-responsive endpoint
3. Conduct a reproductive development and fertility study to determine effects of:
   a. soy infant formula – or –
   b. a mixture of isoflavones in soy infant formula

Proposed Testing Approach:

Specific Aim 1:

PK studies to evaluate developmental profile of equol production in mice

Specific Aim 2:

Uterotrophic assay of soy isoflavones in neonatal mice

Common features of all 3 aims:

- Exposure only during period of lactation
- Direct oral dosing to mouse/rat pup
- Use of glycoslyated soy isoflavones
- Soy infant formula as vehicle

Specific Aim 3:

Evaluation of feasibility to orally administer soy infant formula to rodent pups

yes

Reproductive development and fertility studies with *soy infant formula*
+
PK studies measuring isoflavones

no

Reproductive development and fertility studies with *soy isoflavones*
+
PK studies measuring isoflavones

Pharmacokinetic (PK) Studies of Equol Production during the Period of Lactation in Mice *(Specific Aim 1)*

- Preliminary study to determine blood levels of daidzein
  - Collect blood and assess levels of daidzein, following administration of range of daidzin doses to mouse pups on PND1-5
  - Identify dose level of daidzin resulting in blood levels of daidzein comparable to human infants fed soy infant formula
- Conduct PK studies at different ages during the period of lactation following administration of daidzin to mouse pups
  - Evaluate after single dose with several blood collection timepoints
  - Evaluate after repeated dosing with single blood collection period

Uterotropic Assay in Neonatal Mice *(Specific Aim 2)*

- Mouse pups administered orally a range of doses of isoflavones, individually and in combination, on PND1-5
- On PND5, uteri collected and weighed to measure estrogenic potency of doses to stimulate increase in uterine weight
- Observed mixture data evaluated for evidence of dose addition, response addition, antagonism, or synergism
- If antagonism detected, further mixture experiments evaluate whether daidzin or glycitin inhibit the action of genistin

Evaluation of Feasibility to Administer Soy Infant Formula (*Specific Aim 3*)

- Determine number of feedings necessary to administer 1X soy infant formula to mouse and/or rat pups beginning on PND1
  - Dose volume and handling
- If not feasible to administer 1X soy infant formula, administer a concentrated form of soy infant formula (from powder)
  - Palatability and metabolism issues
- If not feasible to administer soy infant formula (any form), administer a mixture of isoflavones in soy infant formula vehicle
  - Glycosylated isoflavones will be administered in the ratio present in soy infant formula

Reproductive Development and Fertility Study in Rodents (Rats and/or Mice) *(Specific Aim 3)*

- Soy infant formula, or a range of doses of a soy isoflavone mixture, will be administered during the full period of lactation via oral doses to the pups
- Proposed endpoints include:
  - Vaginal opening and time to first estrus
  - Estrous cycling in young and older adulthood (2 vs 6 mo of age)
  - Fertility and fecundity of females
  - Histopathology of reproductive organs in female
  - Mammary hyperplasia in male offspring (rats only)

Significance and Expected Outcomes

- Understanding whether equol is produced in mouse pups during the period of lactation will help better characterize the relevance of the lactational age rodent model for human infants
  - Capacity to produce equol?
- Soy isoflavones are hypothesized to work in a dose-additive manner due to their similar mechanism of action (estrogen receptor agonist)
  - Rodent uterotropic assay is a quick assay of estrogenic potency
- Direct oral dosing of soy infant formula or a mixture of soy isoflavones (as glucosides) to rodent pups during the period of lactation will more closely model human infant exposure to soy infant formula
  - Lactation-only studies are expected to exert similar effects to published reproductive development and fertility studies of genistein/genistin observed on PND 1-5 and in the NTP multigenerational study

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Discussion and Questions

Administering 1X Soy Infant Formula to Mouse and Rat Pups during the Period of Lactation:

Formula intake, human = 1000 mL

Body weight, human (3 mo old) = 6 kg bw

Volume of dose, mouse = 2 µL/g bw

Volume of dose, rat = 5 µL/g bw

Body weight, mouse (PND4) = 2.5 g

Body weight, rat (PND4) = 13.9 g

- Daily formula intake/kg bw , human (3 mo old)

  1000 mL/6 kg bw = 166.7 mL/kg bw = 166.7 µL/g bw

- Daily formula intake, mouse (PND4) = (daily intake/bw human)(bw of PND4 mouse)

  (166.7 µl/g bw)(2.5 g bw) = 416.8 µL

- Daily formula intake, rat (PND4) = (daily intake/bw human)(bw of PND4 rat)

  (166.7 µg/g bw)(13.9 g bw) = 2317.1 µL

- Number of doses needed to administer comparable volume of 1x soy infant formula/ bw as human infant = (daily formula intake)/(dose volume)
  - Mouse on PND4: 416.8 µl / [(2 µl/g bw)(2.5 g bw)] = 83 daily doses
  - Rat on PND4: 2317.1 µl/[(5 µl/g bw)(13.9 g bw)] = 33 daily doses